GENERATION, SAMPLING AND ANALYSIS FOR LOW-LEVEL GB (SARIN) VAPOR FOR INHALATION STUDIES

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ABSTRACT

This study tested and optimized various methodologies to generate, sample and characterize GB test atmospheres in an inhalation chamber, particularly at low vapor levels. A syringe drive/spray atomization system produced GB vapor at lethal concentrations of 1- 44 mg/m^3. A saturator cell was used to generate GB vapor at sub-lethal concentrations down to levels approaching the TLV-TWA of 0.0001 mg/m^3. Both generation techniques demonstrated the ability to produce stable vapor concentrations over extended exposure periods for inhalation toxicology studies. The techniques employed for this study would lay the foundation for testing other chemical warfare agents, such as GF or VX.

INTRODUCTION

Numerous generation, sampling and analytical techniques have been reported for conducting vapor exposures in an inhalation chamber.[1] Vapor generation systems have usually consisted of permeation devices, diffusion cells, liquid injection with heating, and/or direct evaporation of a liquid.[2,3] Vapor collection and analysis techniques have typically used solvent bubblers, solid sorbent tubes and/or gas sample loops followed by gas chromatographic (GC) analysis.

In the past, many of these techniques have been used for inhalation toxicity studies of chemical warfare (CW) agents. These studies have primarily focused on lethal effects that required high vapor concentrations for short-term exposures. However, concerns about worker health and safety, Gulf War syndrome, and physical protective measures (protective masks, clothing, detectors) have prompted a renewed emphasis on the effects of low-level agent exposures. [4]

This study tested various vapor generation, sampling and analysis systems to assess different levels of toxicity (low to high vapor concentrations) in an inhalation chamber. In particular, the system needed to generate stable low GB vapor concentrations approaching the TLV-TWA of 0.0001 mg/m^3. A good starting point for developing this system was to use the nerve agent GB (sarin). GB has a higher volatility compared to the other agents, subsequently this system would help lay the foundation for testing less volatile agents such as GF or VX.

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MATERIALS AND METHODS

CHEMICALS

Chemical agent standard analytical reagent material (CASARM)-grade Sarin (GB) (lot # GB-U-6814-CTF-N (GB2035) was verified as 97.2 $\pm$ 0.2 wt % (as determined by quantitative NMR ^{31}P) in sealed ampules containing nitrogen. The majority of impurities consisted of 0.2% o,o'-diisopropyl methylphosphonate (DIMP), 0.2 % methylphosphonic difluoride (DF), 0.3% methylphosphonofluoridic acid (Fluor Acid), and 0.3% excess HF/F ion. Impurity percentages were based on mole ratios from acid-base titration.

GB TEST ATMOSPSHERE, OVERVIEW

The vaporization system (syringe drive or saturator cell) was contained in a generator box, which in turn was connected to the inlet of a dynamic flow inhalation chamber. High vapor concentrations in the chamber (2 – 44 mg/m^3 GB) were generated with a syringe drive/spray atomization system. Low vapor concentrations (0.0002 – 0.10 mg/m^3 GB) were generated using a saturator cell. The GB vapor was monitored in the chamber with sorbent tube sampling followed by thermal desorption and gas chromatographic (GC) analysis. A phosphorus analyzer also continuously monitored GB vapor at levels exceeding 0.005 mg/m^3.

VAPOR GENERATION SYSTEMS

SYRINGE DRIVE/SPRAY ATOMIZATION SYSTEM

Prior to chamber operation, the liquid GB was drawn into a gas-tight syringe (Hamilton, Reno, NV), transported to the generator box, then mounted onto a variable rate syringe drive (Model 22, Harvard Apparatus Inc., South Natick, MA). Once activated, the syringe drive delivered a constant flowrate of GB (ul/min) through a flexible plastic line (~ 8") into a spray atomization system (Spray Atomization Nozzle 1/4 J SS, Spraying Systems Co., Wheaton Ill) (Fig 1). The atomizer was modified by inserting a syringe needle (SS 25 gauge 3") into the top of the sprayer to decrease the orifice size. As liquid GB entered through the top of the atomizer, compressed air (30-40 psi) entered through the side to atomize the liquid into fine droplets. Due to the volatility of GB, these droplets quickly evaporated into GB vapor, which were then drawn down through the chamber.

SATURATOR CELL

Saturated GB vapor streams were generated by flowing nitrogen carrier gas through a glass vessel, (multi-pass saturator cell) containing liquid GB (Fig 2). The saturator cell consisted of a 100-mm long, 25-mm o.d. cylindrical glass tube with two (inlet, outlet) vertical 7-mm o.d. tubes connected at each end. The main body of the saturator cell contained a hollow ceramic cylinder which served to increase the contact area between the liquid GB and the nitrogen. The saturator cell was fabricated to allow nitrogen to make three passes along the surface of the wetted ceramic cylinder (alundum® fused alumina, Norton Co., Colorado Springs, CO) before exiting the outlet arm of the glass cell. The cell body was also immersed in a constant temperature bath so that a combination of nitrogen flow and temperature could regulate the amount of GB vapor going into the inhalation chamber.

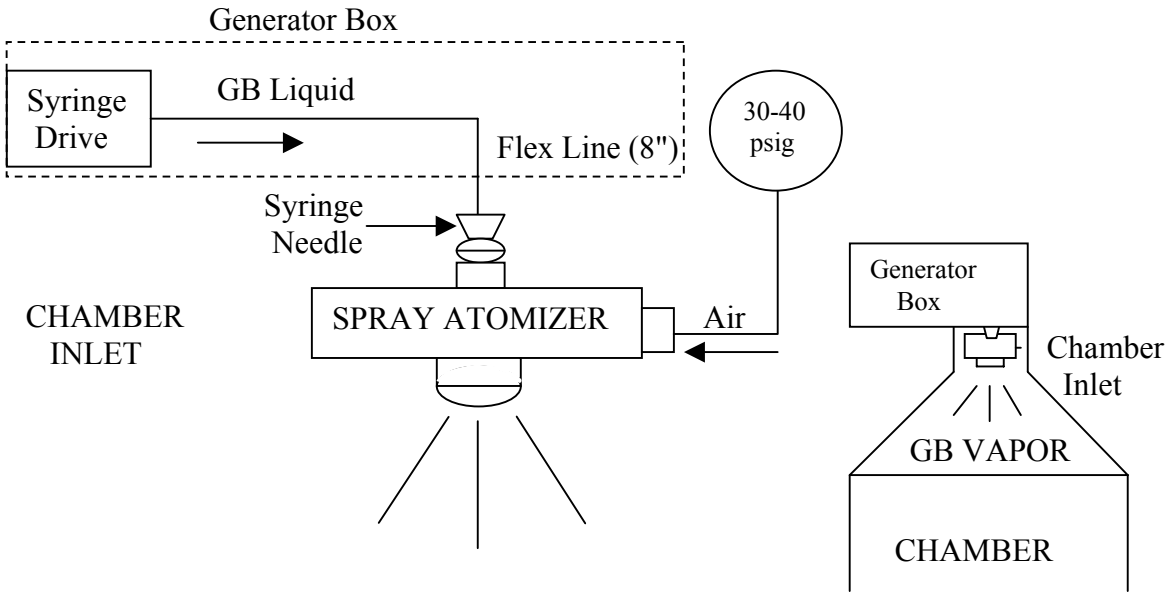

Figure 1. Spray Atomization System.

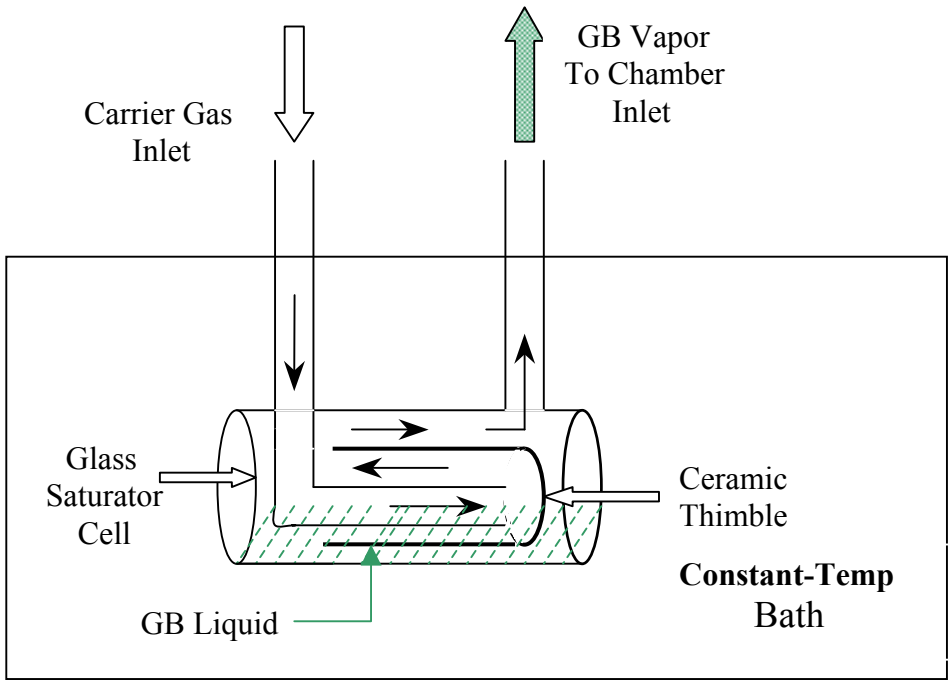

Figure 2. GB vapor Generation via saturator cell.

Typically, the saturator cell was loaded with 2-3 ml of liquid GB (CASARM grade). Immediately after loading, a low nitrogen flowrate (1-2 ml/min) continuously flowed through the cell to maintain the integrity of the liquid GB. This allowed the saturator cell to be used as a generation source for approximately 1-2 weeks.

INHALATION CHAMBER.

GB vapor was monitored in a 750 L dynamic airflow inhalation chamber. The Rochester style chamber was constructed of stainless steel with Plexiglas windows on each of its six sides. The interior of the exposure chamber was maintained under negative pressure (0.25" H_2O), which was monitored with a calibrated magnehelix (Dwyer, Michigan City, IN).

SAMPLING SYSTEM

SORBENT TUBE SYSTEM

The automated solid sorbent tube sampling system consisted of four parts: (1) a heated sample transfer line (2) heated external switching valve (3) thermal desorption unit (Dynatherm) and (4) gas chromatograph (Fig 3). A stainless steel sample line (1/16" o.d. x 0.004" i.d. x 6' length) extended from the middle of the chamber to an external sample valve. From the transfer line, the sample entered a heated (125^o C) 6-port gas-switching valve (UWP, Valco Instruments, Houston, TX). In the by-pass mode, GB vapor from the chamber continuously purged through the sample line and out to a charcoal filter. In the sample mode, the gas sample valve redirected GB vapors from the sample line to a Tenax TA/Haysep sorbent tube (60-80 mesh) located in the Dynatherm (ACEM-900, CDS, Oxford, PA). Temperature and flow programming within the Dynatherm desorbed GB from the sorbent tube directly onto the GC column (RTX-5, 30m, 0.32mm i.d., 1 mm thickness).

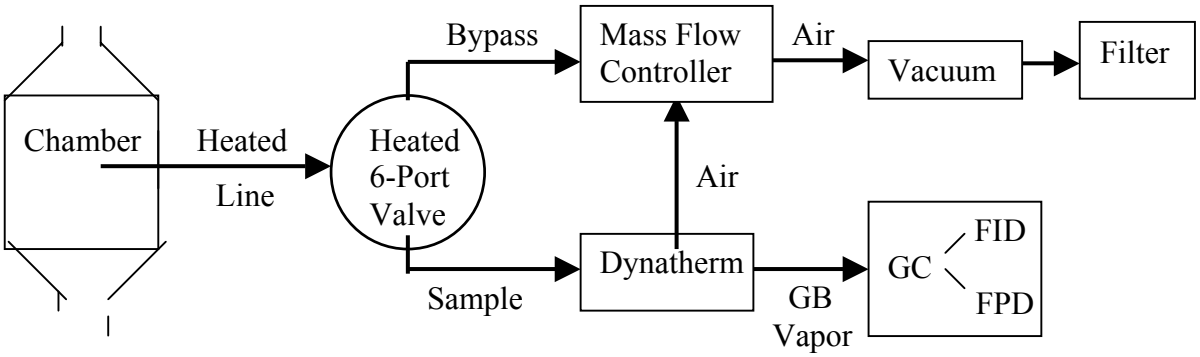

Figure 3. Automated sorbent sampling of GB vapor from the chamber.

The solid sorbent tube sampling system was calibrated by direct injection of external standards (GB/hexane - ug/ml) into the heated sample line of the Dynatherm. In this way, injected GB standards were put through the same sampling and analysis stream as the chamber samples. A linear regression fit ($r^2 = 0.999$) of the standard data was used to compute for the GB concentration of each chamber sample.

PHOSPHORUS MONITOR (HYFED)

GB levels in the chamber (> 0.005 mg/m^3) were continuously monitored with a phosphorus analyzer (HYFED, Model PH262, Columbia Scientific, Austin, Texas). The analyzer output was recorded on a strip chart recorder, which showed the rise, equilibrium, and decay of the chamber vapor concentration during each experimental run.

GENERATION, SAMPLING AND ANALYSIS FOR DIFFERENT LEVELS OF GB VAPOR

GB LEVELS, HIGH RANGE

The spray atomizer was used to generate GB vapor concentrations greater than 1.0 mg/m^3. Syringe drive settings ranged from 1.0 –23 ul/min with chamber flows of approximately 400 – 600 L/min to achieve the vapor concentrations. Once the spray atomizer (~ 30 psi) was activated and the chamber had achieved equilibrium (t_{99}) vapor samples were drawn (0.1 L/min) and collected onto solid sorbent tubes for subsequent GC analysis. In addition to the sorbent tube sampling, the chamber was continuously monitored with a phosphorus analyzer (HYFED) to visualize the chamber profile.

GB LEVELS, MEDIUM RANGE

The saturator cell was used to generate GB vapor concentrations less than 1.0 mg/m^3. Changes in concentration were made primarily through adjustments in water bath temperature and carrier flow through the cell (Table 1). Three separate (4 hr) chamber runs were conducted to evaluate the generator performance at concentrations of 0.01, 0.04 and 0.06 mg/m^3 GB vapor.

Table 1. Generator and Chamber Parameters for 0.01 –0.06 mg/m^3 GB Vapor

GB Vapor (mg/m^3)	**N_2 Flow Through Cell (ml/min)**	**Water Bath Temp (^{o} C)**	**Chamber Flow (L/min)**
0.01	1.0	15	1,618
0.04	4.8	15	1,652
0.06	7.7	16	1,721

GB LEVELS, LOW RANGE

The saturator cell was used to generate low GB vapor concentrations approaching the TLV-TWA of 0.0001 mg/m^3.[5] The primary method to attain these low concentrations was to significantly decrease the water bath temperature for the saturator cell as well as to decrease the carrier flow through the cell. A salt solution (23% sodium chloride dihydrate) was added to the water bath to depress its freezing point .

Three separate chamber runs (4 – 60 hrs) were conducted to evaluate the generator performance at concentrations ranging from 0.0002 – 0.0035 mg/m^3. Generator and chamber parameters used to achieve each concentration are listed in Table 2. All samples were drawn at the rate of 0.4 L/min for each concentration.

Table 2. Generator and Chamber Parameters for 0.0002 –0.0035 mg/m^3 GB Vapor

GB Vapor (mg/m^3)	**N_2 Flow Through Cell (ml/min)**	**Water Bath Temp (^{o} C)**	**Chamber Flow (L/min)**	**Run Time (Hr)**
0.00025	0.4	-17.5	1,607	9
0.0015	0.4	5.8	1,607	12
0.0035	1.0	5.8	1,607	4

RESULTS

GB concentrations determined by sorbent tube GC analysis were plotted over time for each chamber run. Figure 5 summarizes the stability and range of the spray atomizer for three chamber runs at the high GB levels (6 – 44 mg/m^3). Typically this range was used to determine inhalation toxicity for lethality. Figure 6 summarizes the stability and range of the saturator cell for three chamber runs at the medium GB vapor levels (0.01 – 0.06 mg/m^3). Typically this range was used to access inhalation toxicity for sublethal effects ie., miosis.

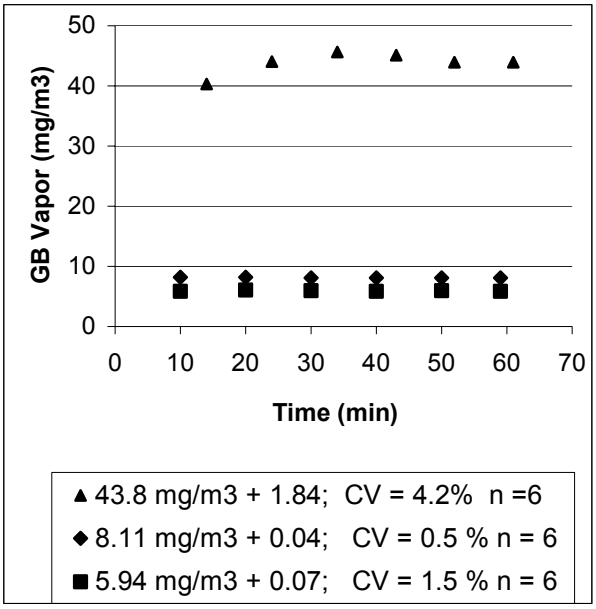

Figure 5. Spray atomizer generation of GB vapor at the high range (6-44 mg/m^3).

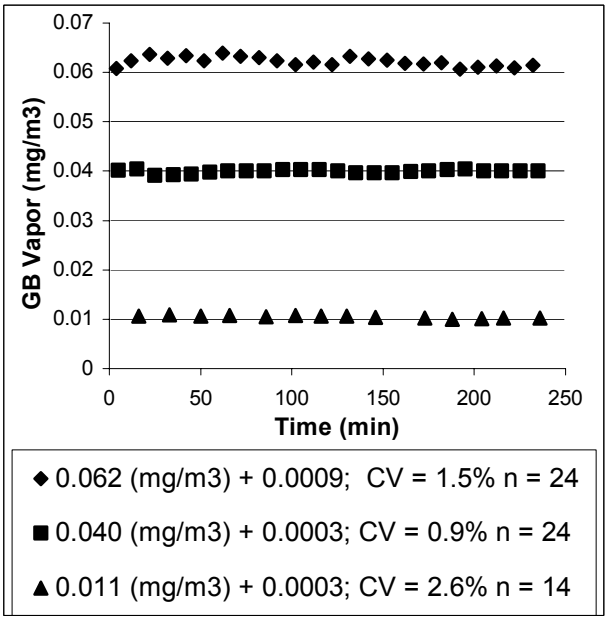

Figure 6. Stability of the saturator cell to generate GB vapor at the medium range.

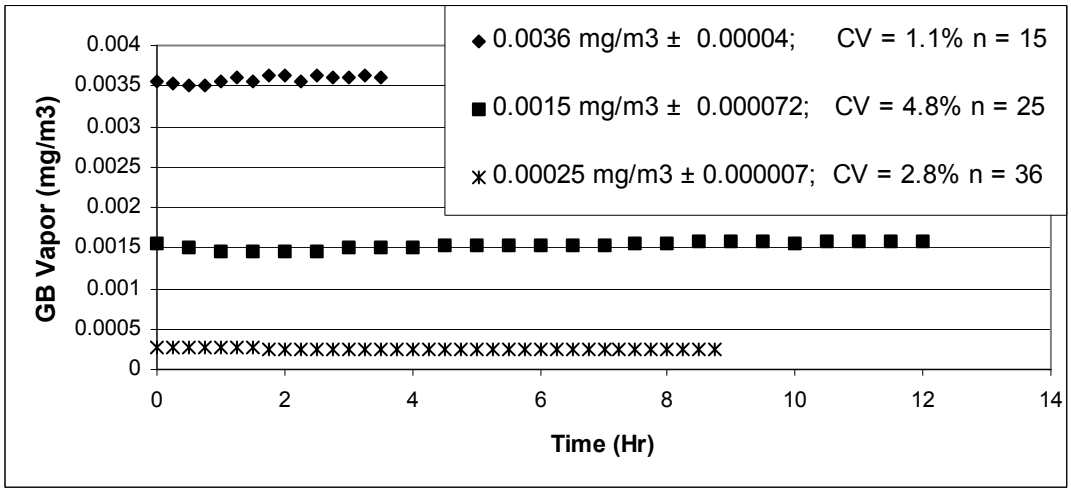

Figure 7. Stability of the saturator cell to generate GB vapor at the low range.

DISCUSSION

SPRAY ATOMIZER

The air atomizer generation technique had been used successfully for the vaporization of dilute GB in hexane and therefore, was tested for the vaporization of neat GB.[6] In this technique, the combination of pressure and orifice size were important parameters to ensure that vaporization was complete and that aerosols (identified by aerosol analyzers, or filter samples) were not formed in the chamber. One advantage of this system over heating methods was that it did not alter the characteristics of the chemical. In other words, once the spray atomization vaporized the agent in the chamber, the agent did not recondense back into an aerosol. Conversely, an agent that had been vaporized through heating had the potential to recondense back into an aerosol once it had hit the cooler temperature in the chamber.

SATURATOR CELL

The saturator cell was originally used as a means to generate stable vapor concentrations to determine the vapor pressure of VX and DMMP at various carrier flows and temperatures.[7] An extension of this capability was to use it as a continuous vapor source on an inhalation chamber.

The saturator cell, in conjunction with the chamber flow, has generated a range of 0.00025 – 0.5 mg/m^3 GB vapor in this and other studies.[6] This generator has a useful range extending to three orders of magnitude. It operated efficiently with a small amount of agent (2-3 ml) and continuously for 1-2 weeks of operation. This also allowed for chamber passivation (conditioning of chamber to adsorption of GB on inner chamber surfaces) to occur in-between exposures. Chamber conditioning time was significantly greater at the low level than the higher concentrations.

GB MIOSIS EXPOSURES

The low GB concentrations (.0002-0.5 mg/m^3) typically represented the toxicity range for subclinical signs or for miosis (extended exposure). Mioduszewski et al., conducted GB vapor exposures (0.01 – 0.5 mg/m^3) with the saturator cell for 10 min to 4 hrs to determine miosis thresholds in rats.[8] Van Helden et al., conducted low-level acute exposures for 5 hr to examine the lowest observable effects of GB exposure in guinea pigs and marmosets.[9] Miosis was observed at exposure concentrations ranging from 0.0075 - 0.15 mg/m^3 for guinea pigs and .0073 - 0.138 mg/m3 for marmosets.[9] These concentrations were well within the range for the low-levels tested in this study. In addition, the percent variance for all the chamber runs in this study was within 1-5%.

CONCLUSIONS

This paper describes techniques that were successful for the generation, sampling and analysis of GB vapor at various toxicological significant levels. The spray atomization system was an effective generator for high (lethal) GB vapor concentrations at a range of 1-50 mg/m^3. The saturator cell generator was most effective at sub-lethal concentrations and had a large effective range from 0.00025 - 0.1 mg/m^3 GB. Both generators produced stable vapor concentrations for an extended period of time with variations ranging from 1-5%. The sampling and analysis system was a sensitive method for low-level GB vapor. These techniques should be useful for testing less volatile agents such as GF and VX.

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